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Infectious complications in patients with acute myeloid leukemia treated according to the protocol with daunorubicin and cytarabine with or without addition of cladribine. A multicenter study by the Polish Adult Leukemia Group (PALG)☆

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Summary
Objectives: The addition of cladribine to the standard regimen consisting of daunorubicin and cytarabine has been reported to increase the efficacy of induction therapy in acute myeloid leukemia (AML). The goal of this study was to determine the effect of this modification on the incidence and spectrum of infectious complications.
Methods: Case report forms of 309 patients with newly diagnosed AML who had been enrolled in the prospective, randomized 'DAC-7 vs. DA-7' trial were reviewed. The frequency, etiology, localization, severity, and outcome of infections were compared for patients receiving only daunorubicin and cytarabine (DA-7) and those additionally treated with cladribine (DAC-7).
Results: A total of 443 febrile episodes were reported with no significant difference between the treatment groups. A trend towards a higher frequency of bacteremias was observed among DA-7 patients compared to those in the DAC-7 group (31% vs. 21%; $p = 0.08$). The treatment arms did not differ in terms of the distribution of the isolated Gram-positive, Gram-negative, fungal, and viral organisms. However, when bacteremias were considered, Gram-positive blood cultures tended to be more frequent in the DA-7 compared to the DAC-7 group (16% vs. 8.5%; $p = 0.07$). This difference reached statistical significance when major blood bacteremias were analyzed separately (13% vs. 5%; $p = 0.02$). Complete recovery from infections was observed in the majority of patients across both treatment arms and no significant difference was noted regarding infection-related mortality.
Conclusions: The addition of cladribine to standard induction chemotherapy has no impact on the incidence and spectrum of infectious complications in newly diagnosed AML patients.

Introduction

Patients with acute myeloid leukemia (AML) who undergo intensive chemotherapy are at high risk for infectious complications. Serious, life-threatening infections remain a major cause of morbidity and mortality in this group of patients.[1] Several risk factors, mainly profound neutropenia with a neutrophil count $<0.1 \times 10^9$/l lasting more than 10 days, have been reported to increase the susceptibility to infections in AML patients.[1,2] In addition, other risk factors such as impaired cellular and humoral immunity caused by the underlying disease, severe mucositis, the degree of hemorrhagic diathesis of skin and mucosal tissue, as well as a number of skin-penetrating venous catheters are considered to predispose to infections.[2–4]

An induction chemotherapy consisting of daunorubicin (DNR) for 3 days and cytarabine (Ara-C) for 7 days (DA-7 regimen) remains the standard first-line treatment in newly diagnosed AML patients aged less than 60 years.[5–7] The results of AML therapy have improved in recent years due to the intensification of Ara-C dose and the application of new drugs to the induction treatment.[8,9] Purine nucleoside analogs (PNAs), such as cladribine and fludarabine, commonly used in the therapy of indolent lymphoid disorders, have recently been introduced in the therapy of myeloid malignancies.[7,10–13] The immunosuppressive effect of PNAs as well as the increased incidence of related infections have been reported for patients with lymphoproliferative disorders.[14–22] The pathogenesis of this phenomenon seems to be complex, multifactorial, and only partly understood. It is known that PNAs can induce severe, prolonged immunosuppression due to long-lasting defects in cell-mediated immunity with a decrease in the CD4+/CD8+ ratio. The PNA therapy may also result in bone marrow suppression with prolonged neutropenia, anemia, and thrombocytopenia.[14–18] In contrast, for AML patients data are scarce, since the majority of published studies have mainly focused on PNA efficacy.[7,10–13,23–27] In a previous study we demonstrated that the addition of cladribine (2-CdA) to the DA-7 induction regimen increased the efficacy of chemotherapy, resulting in a higher complete remission rate observed after a single course of treatment.[7] However, until now, it has not been determined whether this enhanced anti-leukemic effect is associated with an increased incidence of infectious complications. Therefore, verification of this hypothesis was the scope of the current study. We addressed the question of whether or not the addition of 2-CdA to the DA-7 regimen influences the incidence and spectrum of infections during the period of induction chemotherapy in newly diagnosed AML patients.

Patients and methods

Study design

We reviewed the case report forms of 309 patients with newly diagnosed AML who had been enrolled in the prospective, multicenter, randomized 'DAC-7 vs. DA-7' trial between May 1999 and June 2002.[7] This study was performed in 11 centers of the Polish Adult Leukemia Group (PALG) and was approved by the local ethics committees. Informed consent was obtained from all patients, and patient confidentiality was preserved in accordance with the Polish regulations for studies of human subjects. Inclusion criteria for the 'DAC-7 vs. DA-7' protocol were as follows: (1) AML diagnosis established according to the French–American–British (FAB) classification, (2) immune phenotyping performed, and (3) age 16–60 years. Patients with FAB M3 subtype confirmed by promyelocytic leukemia (PML)/retinoic acid receptor-alpha (RAR-α) rearrangement and/or t(15;17), as well as those with poor performance status (Karnofsky index $<40\%$), preceding chemo- or radiotherapy, severe organ impairment, or pregnancy were not eligible for the study.

Treatment

Patients were centrally randomized to one of the two following induction treatment arms: (1) DAC-7, which consisted of DNR 60 mg/m^2 IV on days 1–3, Ara-C 200 mg/m^2 CV (central venous) on days 1–7, and cladribine (Biodribin, Bioton, Warsaw, Poland) 5 mg/m^2 IV on days 1–5, and (2) DA-7, which consisted of DNR and Ara-C in the same schedule without cladribine. The comparison of the efficacy of the two induction regimens was the subject of a previous report.[7]

Antimicrobial and supportive therapy were given to the patients according to commonly accepted guidelines.[28] In the case of the appearance of febrile episodes or infection symptoms, empirical antimicrobial therapy was administered according to the local variations in the frequency of isolated pathogens and their drug-resistance pattern. Empirical first-line antibiotic therapies were categorized into two groups: monotherapy consisted of cephalosporin, penicillin, or carbapenem; and two-drug therapy comprised cephalosporin/anti-pseudomonal penicillin/carbapenem + aminoglycoside or ciprofloxacin + amoxicillin–clavulanate. Vancomycin was added, if fever persisted for 48–72 hours and at least one of the following clinical findings was met: clinically suspected serious catheter-related infections, known colonization with penicillin- and cephalosporin-resistant pneumococci or methicillin-resistant *Staphylococcus aureus*, positive blood cultures for Gram-positive bacteria before the final identification, hypotension or other evidence of cardiovascular impairment.[29] Amphotericin B was given empirically if the patient did not respond to antibiotic therapy within 5 days. Empirical therapy was given to those febrile patients for whom there was yet no microbiological explanation for fever. Cultures from blood and other suspected sites of infection were collected from all febrile patients. Modifications of the antibiotic therapy were made on the basis of results of cultures and susceptibilities of microorganisms to antimicrobial agents. All antibiotics were discontinued after the patient had been afebrile for 3–5 days and neutrophil recovery occurred.

Assessment of infection incidence

Each episode of fever or the presence of infection symptoms was reviewed and the series of data were collected for each discrete infection. Fever was considered as a single temperature measurement of $\geq$38.3 °C or $\geq$38.0 °C over at least 1 hour, in the absence of obvious environmental causes.[28,29] All suspected or documented infections were graded according to the National Cancer Institute's Common Toxicity Criteria.

The causative agent, when one was identified, was classified as bacterial (Gram-positive, Gram-negative, or other), fungal (*Candida* species, *Aspergillus* species, or other), or viral (varicella zoster virus, herpes simplex virus).

The site of infection was classified as oral cavity infections (stomatitis) and upper respiratory tract (pharyngitis, sinusitis, rhinitis), lower respiratory tract (pneumonia or bronchitis), gastrointestinal (GI) tract, urinary tract, blood (bacteremia), skin and/or soft tissues, and other. Blood cultures were categorized as monomicrobial or polymicrobial, the latter if more than one organism was isolated in the same blood culture, or in a separate blood culture obtained within 24 hours.[30,31] Catheter-related bloodstream infections were defined as those infections in which at least one of the following conditions was met: (1) fever (>38.0 °C) with chills and rigors within 1 hour after catheter flushing or manipulation; (2) isolation of a pathogen from a blood culture drawn through the catheter, but not from another blood culture drawn from a peripheral vein at the same time; (3) isolation of the same pathogen from the catheter tip and from blood; (4) isolation of the same organism both from blood and from purulent material drained from the catheter exit site or from the subcutaneous tunnel.[31,32] Fever of unknown origin (FUO) was defined as isolated fever without an identifiable focus of infection. If the site of infection was later defined in the clinical course of the patient, the infection was categorized as being from that site and not as an FUO.

Neutropenia was defined as an absolute neutrophil count (ANC) less than 1.0×10^9/l.[28,29] ANC was further categorized according to whether they were $<0.5 \times 10^9$/l or $<0.1 \times 10^9$/l at the onset of febrile episodes. Laboratory data collected at the time of infection appearance included white blood cell (WBC) count, ANC defined as the number of segmented neutrophils and bands, the lymphocyte count, and serum creatinine concentration.

Assessment of infection outcome

The outcome of infections for each patient was determined at the completion of the antimicrobial therapy. All patients who died of infection were classified as a failure. Complete response to infection was defined as complete resolution of all clinical and microbiological signs and symptoms of infection except of pneumonia. In the latter case, complete response was defined as resolution of all symptoms and significant improvement demonstrated by chest radiographs or computed tomography (CT) scans. Partial response to infection was classified as significant improvement of clinical symptoms, negativity of all cultures, and/or partial regression of pulmonary infiltrates in chest roentgenography. Additionally, time of IV administration of antibiotics was used to assess the effectiveness of antimicrobial therapy.

Statistical analysis

The incidence of infections during induction therapy was the primary study end-point. Secondary end-points were: spectrum of infection (e.g., causative agent or site of infection), absolute neutrophil and lymphocyte counts at the time of infection occurrence, duration of anti-infectious therapy, duration of supportive therapy with hematopoietic growth factors, and infection outcome.

The two-tailed Pearson's Chi-square test and Fisher's exact test were used to test the statistical significance of differences in categorical data. For continuous variables the Student's *t*-test and the Mann–Whitney U-test were used when appropriate. The significance level (alpha) of 0.05 was used for all statistical tests, and no attempt was made to adjust the alpha level for the large number of statistical tests performed for this study. Thus, some tests recognized as significant may be a result of chance alone, and this fact should be kept in mind when interpreting as significant test statistics with a *p*-value only slightly smaller than 0.05.

Table 1 Characteristics of 309 acute myeloid leukemia patients treated with DAC-7 or DA-7 induction chemotherapy

	DAC-7 (N = 152)	DA-7 (N = 157)	p-Value
Age, years	44 (16–60)	45 (17–60)	0.6
Sex, male/female	80/72	78/79	0.6
AML subtypes			
M0	5 (3.3)	6 (3.8)	1.0
M1	36 (23.7)	35 (22.3)	
M2	41 (27.0)	47 (29.9)	
M4	53 (34.9)	52 (33.1)	
M5	12 (7.9)	12 (7.6)	
M6	4 (2.6)	4 (2.5)	
M7	1 (0.6)	1 (0.6)	0.2
Central venous catheter	102 (67.1)	113 (72.0)	0.4
Creatinine concentration at infection manifestation (mg/dl)	0.86 (0.4–1.6)	0.9 (0.1–1.72)	0.1
WBC at infection manifestation $\times 10^9$/l	0.8 (0.1–191)	1.05 (0.1–286)	0.08
ANC at infection manifestation $\times 10^9$/l	0.1 (0–9.4)	0.2 (0–23)	
Lymphocytes at infection manifestation $\times 10^9$/l	0.5 (0–19)	0.66 (0.04–37)	0.007

Results are median (range) or n (%).
AML, acute myeloid leukemia; M0, AML minimally differentiated; M1, AML without maturation; M2, AML with maturation; M4, acute myelomonocytic leukemia; M5, acute monoblastic and monocytic leukemia; M6, acute erythroid leukemia; M7, acute megakaryoblastic leukemia; WBC, white blood cell; ANC, absolute neutrophil count.

Results

Patient characteristics

Demographic and clinical data for 309 AML patients assessable for infectious complications are presented in Table 1. One hundred fifty-two patients were treated with DAC-7 induction chemotherapy and 157 patients received the DA-7 regimen. The two treatment groups did not differ in terms of age, gender, AML subtypes, preceding myelodysplastic syndrome (MDS), the proportion of patients with a central venous catheter, as well as median of creatinine concentration, WBC and ANC counts at the time of infection occurrence. The majority of patients had a neutropenia with ANC $<1.0 \times 10^9$/l (86% in DAC-7 vs. 86% in DA-7; $p = 1.0$), or ANC $<0.5 \times 10^9$/l (75% in DAC-7 vs. 74% in DA-7; $p = 0.8$) at the onset of infection; however, only a half of patients showed a profound neutropenia with ANC $<0.1 \times 10^9$/l (54% in DAC-7 vs. 45% in DA-7; $p = 0.2$). The median number of days with grade IV neutropenia was 18 (range 7–37) in the DAC-7 group and 19 (range 5–34) in the DA-7 group ($p = 0.1$). The patients who received the DAC-7 regimen presented significantly more profound lymphocytopenia compared to those treated with DA-7 chemotherapy at the time of infection manifestation ($p = 0.007$) (Table 1). Data concerning the duration of lymphocytopenia were not collected during this study.

Prophylactic antibacterial antibiotics were administered to 224 (72%) of all AML patients starting on the first day of chemotherapy and stopping after the recovery of ANC $>0.5 \times 10^9$/l. Of these patients, 49% received trimethoprim–sulfamethoxazole, 26% penicillins, and 20% of patients received quinolones. There were no differences in the usage of antibacterial prophylaxis ($p = 0.8$) or the type of antibiotics used ($p = 0.8$) between the DAC-7 and DA-7 patients. The majority of patients ($n = 283$, 92%) received antifungal prophylaxis. Azoles, mainly fluconazole, were administered to 90% of these subjects. Similarly, no differences were observed in the usage of antifungal prophylaxis ($p = 0.6$) or the type of antifungal drugs used ($p = 1.0$) between patients treated with the DAC-7 and DA-7 regimens.

Infection incidence

A total of 443 febrile episodes and infections occurred in 309 patients over the induction treatment period with the same frequency in DAC-7 and DA-7 patients (93% vs. 90%; $p = 0.4$). The mean number of infections per patient was similar in the two treatment arms (Table 2). Over the induction treatment

Table 2 Frequency of infections among acute myeloid leukemia patients according to the treatment arm

	DAC-7 (N = 152)	DA-7 (N = 157)	p-Value
	All infections		
Total number of infections	220	223	
Number of patients with any infection (%)	141 (93)	141 (90)	0.4
Mean number of infections per patient	1.44 ± 0.77	1.40 ± 0.76	0.9
	Major infections		
Total number of major infections	76	92	
Number of patients with a major infection (%)	64 (42)	68 (43)	0.7
Mean number of major infections per patient	0.53 ± 0.65	0.64 ± 0.76	0.3

Table 3 Site of infection in acute myeloid leukemia patients according to the treatment arm

	All infections			Major infections		
	Patients with at least one infection, *n* (%)			Patients with at least one infection, *n* (%)		
	DAC-7	DA-7	*p*-Value	DAC-7	DA-7	*p*-Value
Oral cavity	44 (31)	41 (29)	0.8	9 (6)	7 (5)	0.8
Upper respiratory tract	22 (16)	20 (14)	0.9	3 (2)	1 (0.7)	0.6
Lower respiratory tract	43 (30)	37 (26)	0.5	23 (16)	27 (19)	0.6
Gastrointestinal tract	9 (6)	11 (8)	0.8	4 (3)	5 (3.5)	1.0
Urinary tract	5 (3.5)	3 (2)	0.7	1 (0.7)	1 (0.7)	1.0
Blood	30 (21)	44 (31)	0.08	23 (16)	38 (27)	0.04
Skin/soft tissue	9 (6)	13 (9)	0.5	0	0	-
FUO	52 (37)	45 (32)	0.4	11 (8)	8 (6)	0.6
Venous catheter	4 (3)	7 (5)	0.5	2 (1)	3 (2)	1.0
Other sites	2 (1)	2 (1)	1.0	0	2 (1)	0.5

FUO, fever of unknown origin.

period, 168 major infections (grade 3 or 4 according to the World Health Organization classification) developed with the same frequency in patients treated with the DAC-7 and DA-7 regimens (42% vs. 43%; $p = 0.7$). There was no significant difference between the groups in terms of the number of major infections (Table 2).

Site of infection

The sites of all infectious complications and major infections are listed in Table 3. Across the two treatment arms, infections of the oral cavity and upper respiratory tract were most frequent and developed in 47% of DAC-7 and 43% of DA-7 patients ($p = 0.8$). More than one-third of patients from both groups developed FUO (37% in the DAC-7 arm vs. 32% in the DA-7 arm; $p = 0.4$). Eighty cases of lower respiratory tract infection, mainly pneumonias, were observed during the induction chemotherapy period, with similar frequency in both groups (30% in DAC-7 vs. 26% in DA-7; $p = 0.5$). The rate of positive results of blood cultures was 26% in all AML patients during the induction treatment period. Overall, 74 episodes of bacteremia were reported and a trend towards a higher incidence was observed among DA-7 patients compared to DAC-7 patients (31% vs. 21%; $p = 0.08$). In the analysis restricted solely to major infectious complications, the difference in the incidence of bacteremia was marginally significant (27% vs. 16%; $p = 0.04$). Of 74 bacteremia episodes, 50 (67%) occurred in patients fitted with a central venous catheter. Catheter-related infections accounted for 11 of 50 (22%) bacteremia episodes. There was no difference in the proportion of catheter-related infections according to the treatment regimen (3% in DAC-7 vs. 5% in DA-7; $p = 0.5$).

Causative agent

A causative agent of infection was documented in 214 (48%) of 443 febrile episodes, and in 130 (77%) of the major infections (Table 4). Of the 214 episodes, 65 (30%) were due to Gram-positive bacteria, 36 (17%) to Gram-negative bacteria, and 31 (14%) were polymicrobial infections. In addition, 43 episodes (20%) were caused by proven fungal infections. Of these fungal infections, 36 (84%) were caused by *Candida* species, and only seven (16%) episodes were due to *Aspergillus* species. In the study population, 33 (15%) viral infections were observed, and the majority of them were caused by herpesvirus. As shown in Table 4, there were no statistically significant differences in the distribution of Gram-positive, Gram-negative, fungal, or viral organisms among patients treated with the DAC-7 or DA-7 regimen. However, a trend towards a higher incidence of major Gram-

Table 4 Causative microorganisms identified during infections in acute myeloid leukemia patients

	All infections			Major infections		
	Patients with at least one infection, *n* (%)			Patients with at least one infection, *n* (%)		
	DAC-7	DA-7	*p*-Value	DAC-7	DA-7	*p*-Value
Gram-positive bacteria	29 (20.5)	36 (25.5)	0.4	15 (11)	26 (18)	0.09
Gram-negative bacteria	14 (10)	22 (16)	0.2	9 (6)	15 (11)	0.3
Polymicrobial bacteria	19 (13)	12 (8.5)	0.2	13 (9)	9 (6)	0.5
Anaerobic bacteria	2 (1)	3 (2)	1.0	2 (1)	1 (0.7)	1.0
Mycobacterium tuberculosis	0	1 (0.7)	1.0	0	1 (0.7)	1.0
Candida species	21 (15)	15 (11)	0.4	11 (8)	9 (6)	0.8
Aspergillus species	5 (3.5)	2 (1)	0.4	5 (3.5)	1 (0.7)	0.2
Varicella zoster/herpes simplex virus	16 (11)	17 (12)	1.0	8 (6)	5 (3.5)	0.6

Table 5 Frequency of any bacteremia or fungemia in acute myeloid leukemia patients according to the treatment arm

	All infections			Major infections		
	Patients with at least one infection, *n* (%)			Patients with at least one infection, *n* (%)		
	DAC-7	DA-7	*p*-Value	DAC-7	DA-7	*p*-Value
Gram-positive bacteremia	12 (8.5)	23 (16)	0.07	7 (5)	19 (13)	0.02
Gram-negative bacteremia	6 (4)	11 (8)	0.3	6 (4)	9 (6)	0.6
Polymicrobial bacteremia	10 (7)	7 (5)	0.6	8 (6)	7 (5)	1.0
Fungemia	2 (1)	3 (2)	1.0	2 (1)	3 (2)	1.0

positive infections in patients receiving the DA-7 regimen compared to those treated with the DAC-7 regimen was observed. One case of infection due to *Mycobacterium tuberculosis* was reported in a patient treated with DA-7 chemotherapy.

A separate analysis was conducted for episodes with positive blood cultures (Table 5). Microbiological analysis revealed that Gram-positive cocci caused 47% of bacteremias, Gram-negative rods caused 23% of bacteremias, and 23% of bacteremias were polymicrobial. Five episodes of fungemia (7%) were observed in AML patients during the induction treatment period. All fungemias were caused by *Candida* species. Comparing the two treatment arms, Gram-positive blood cultures were reported more frequently in patients treated with the DA-7 regimen compared to those receiving DAC-7 therapy, however the difference was not statistically significant. This tendency was more pronounced for major infections analyzed separately (13% vs. 5%; $p = 0.02$). The detailed distribution of organisms in the 74 episodes of bacteremia and fungemia is shown in Table 6. Across the two treatment arms, coagulase-negative staphylococci were the most frequently isolated pathogens (21/74; 28%), followed by polymicrobial organisms (17/74; 23%) and *Staphylococcus aureus* (7/74; 9%). Other pathogens were isolated with similar frequencies, including enterococci (5/74; 7%), *Pseudomonas* species (5/74; 7%), and *Candida* species (5/74; 7%).

Infection outcome

Complete recovery from infectious complications was observed in the majority of patients across the two treatment arms (84% in the DAC-7 group and 84% in the DA-7 group; Table 7). During the induction treatment period, 31 (11%) patients died due to infectious complications. They did not respond to antimicrobial therapies and the majority developed septic shock. Bacteremia episodes were complicated by septic shock in 14 patients, with a similar distribution in both

Table 6 Distribution of organisms among acute myeloid leukemia patients with positive blood cultures according to the treatment arm

	Patients with at least one episode of bacteremia or fungemia		
Organisms	DAC-7 ($n = 30$)	DA-7 ($n = 44$)	*p*-Value
Gram-positive organisms			
Staphylococcus aureus			
Methicillin-susceptible	1 (3)	2 (4.5)	1.0
Methicillin-resistant	2 (7)	2 (4.5)	1.0
Coagulase-negative Staphylococcus			
Methicillin-susceptible	3 (10)	4 (9)	1.0
Methicillin-resistant	4 (13)	10 (23)	0.4
Enterococcus species	2 (7)	3 (7)	1.0
Corynebacterium species	0	2 (4.5)	0.5
Gram-negative organisms			
Escherichia coli	1 (3)	2 (4.5)	1.0
Pseudomonas species	1 (3)	4 (8.5)	0.6
Klebsiella species	1 (3)	1 (2)	1.0
Enterobacter species	0	2 (4.5)	0.5
Acinetobacter species	2 (7)	2 (4.5)	1.0
Serratia marcescens	1 (3)	0	1.0
Polymicrobial	10 (33)	7 (16)	0.1
Fungi			
Candida species	2 (7)	3 (7)	1.0

Table 7 Infection outcome in acute myeloid leukemia patients according to the treatment arm

Response to antimicrobial therapy	Patients with at least one infection, *n* (%)			Patients with at least one major infection, *n* (%)		
	DAC-7 (*n* = 141)	DA-7 (*n* = 141)	*p*-Value	DAC-7 (*n* = 64)	DA-7 (*n* = 68)	*p*-Value
Complete response	119 (84)	119 (84)	0.2	45 (70)	51 (75)	0.3
Partial response	2 (1)	6 (4)		2 (3)	5 (7)	
Infection-related death	18 (13)	13 (9)		17 (27)	12 (18)	

treatment arms. All these patients had neutropenia $<1.0 \times 10^9$/l at the onset of infection. No significant difference in infection-related mortality was observed among the two treatment groups.

Five patients (two subjects from the DAC-7 group and three from the DA-7 group) were not considered for infection outcome assessment because they died due to other complications, including four with central nervous system hemorrhage and one patient with myocardial infarction.

Two hundred eighty-two patients (91%) required empirical first-line antibiotic therapy. Of these, 233 (83%) required the addition of a new antibacterial drug or complete replacement of the empirical first-line regimen. No difference was noted with regard to the necessity for first-line antibiotic change between patients receiving DAC-7 or DA-7 (78% vs. 76%; $p = 0.8$). Of 282 patients who had received antimicrobial therapy, empirical antifungal therapy was given to 123 (44%) patients. Similarly, there was no statistical difference concerning the application of antifungal antibiotics among DAC-7 and DA-7 patients (44% vs. 40%; $p = 0.4$). The median duration of antimicrobial IV therapy was marginally longer in the DAC-7 patients than the DA-7 patients (25 days, range 0—62 days and 24 days, range 0—74 days, respectively; $p = 0.054$). In the separate analysis of major infections, the duration of IV antibiotic therapy was similar in both treatment arms (28 days, range 4—62 days in the DAC-7 group and 27 days, range 6—74 days in the DA-7 group; $p = 0.2$).

The use of supportive therapy with hematopoietic growth factors was comparable in both study groups. Thirty-six patients (25%) in the DAC-7 arm and 33 patients (23%) in the DA-7 arm received granulocyte colony-stimulating factor (G-CSF) to enhance ANC recovery during infectious complications. The median therapy with G-CSF was 7 days (range 1—16 days) in the DAC-7 group compared to 6 days (range 2—20 days) in the DA-7 group ($p = 0.6$).

Discussion

The results of the present study did not reveal any significant differences regarding the frequency, severity, or spectrum of infectious complications in AML patients treated with DA-7 or DAC-7 regimens during the period of induction chemotherapy. Additionally, the microbiological analysis of overall infection episodes showed no significant differences in the distribution of Gram-positive, Gram-negative, fungal, or viral organisms isolated from patients treated with DAC-7 or DA-7 regimens. Since this was a retrospective study, any assumption about the number of patients in the particular groups was not performed. With respect to this, the lack of power as a potential source of error should be taken into consideration. Interestingly, the only difference noted was the incidence of bacteremias: Gram-positive bacteria were unexpectedly reported more frequently in patients receiving the DA-7 regimen compared to those receiving the DAC-7 regimen. This difference reached statistical significance when major infections were analyzed separately. However, it should be underlined that these results also need to be interpreted with caution due to the large number of multiple statistical comparisons performed for this study.

The overall incidence as well as profile of infections in the whole study population was consistent with previously published reports on AML patients undergoing induction therapy.[6,33—36] In our study, 91% of patients presented any infectious complication, and serious infections were observed in approximately 43% of patients. Over 80% of patients with neutropenia after chemotherapy have been reported to develop fever.[2,28,29] As the source of febrile episodes is often difficult to identify, about 30% of infections were considered as FUO.[2] In the present study, at least one site of fever was identified in 78% of all febrile episodes, whereas 22% of infectious complications were expressed as FUO. Our results are comparable with those observed by Madani, who identified a source of fever in 81% of infections and 19% of febrile episodes classified as FUO.[33] The author showed that in AML patients, the most common cause of fever was mucositis followed by pneumonia, central venous catheter infection, neutropenic enterocolitis, and invasive fungal disease.[33] In our study, the distribution of infection sites was similar; the oral cavity, mainly related to mucositis, and the upper respiratory tract were the most frequent sites of infection, followed by lower respiratory tract involvement. Infections of the gastrointestinal tract and central venous catheter, as well as skin and soft tissues were observed less frequently in our patients. Bloodstream infections in AML patients undergoing chemotherapy were reported to be associated with 38% of febrile episodes.[33] In the present study, bacteremias were related to 17% (74/443) of all febrile episodes, and the incidence of bloodstream infections among AML patients reached 26% (74/282). This is in line with results reported by Klastersky et al. who observed that 23% of cancer patients with febrile neutropenia developed bacteremia.[37]

Over the last two decades, a shift towards a higher frequency of Gram-positive infections has been observed, whereas Gram-negative pathogens are less frequently isolated from neutropenic patients with cancer.[2,29,38,39] This may be as a result, in part, of the use of more intensive chemotherapy, particularly high dose cytarabine, which has contributed to an increased incidence of severe mucositis, profound and prolonged neutropenia, the common application of central venous catheters, and the use of antibiotic prophylaxis, particularly co-trimoxazole and

fluoroquinolones.[38,39] The spectrum of infections reported in our study remains in line with the observations of other investigators.[13,34,35] Bacterial organisms were proven in 76% (138/181) of all microbiologically documented infections. Among these pathogens, Gram-positive bacteria, especially coagulase-negative staphylococci and enterococci, were isolated most frequently. However, we noted an increased incidence of polymicrobial blood cultures as compared to other reports.[37] Viral infections, mainly clinically documented, accounted for 7.5% (33/443) of all febrile episodes in this study. The majority of them were caused by herpesvirus and no case of cytomegalovirus infection was recorded. Fungal infections accounted for approximately 10% (43/443) of all febrile episodes. *Candida* species were the most frequently isolated fungi and caused mainly oral candidiasis. The frequency of fungemia observed in our study was slightly lower (7%; 5/74) compared to that reported by Madani (10.6%).[33] It is known that microbiological confirmation of fungal species presents major clinical problems.[36] It cannot be ruled out that the proportion of fungal infections among our patients would be higher if more invasive diagnostic methods were used to identify the causative agent of infection. However, such diagnostic methods may be difficult to conduct in AML patients, especially in those with profound neutropenia and thrombocytopenia at the onset of infection.

The administration of standard induction chemotherapy consisting of daunorubicin and cytarabine results in profound neutropenia and lymphocytopenia in AML patients. It may be hypothesized that the incorporation of another myelo- and immunosuppressive agent such as cladribine could provide additional risk for life-threatening complications. It has also been reported that chemotherapy regimens consisting of high-dose cytarabine with PNAs lead to profound neutropenia and T-cell depletion, and in consequence contribute to an increased risk for invasive fungal and viral infections.[37] Moreover, the use of PNAs may introduce the potential for a new spectrum of infectious complications.[17,18] Infections caused by *Pneumocystis carinii*, *Listeria monocytogenes*, cytomegalovirus, herpes simplex and varicella zoster virus, as well as *Mycobacterium tuberculosis* and fungi have frequently been observed in patients treated with PNAs.[17–20] In our study, although patients treated with cladribine presented more profound lymphocytopenia at the onset of infection, the frequency and spectrum of infectious complications as well as the infection outcome were comparable in both treatment groups. Our findings could probably be explained by the fact that DAC-7 and DA-7 patients did not differ significantly in terms of duration of neutropenia, which seems to be the most important factor determining susceptibility to infections in AML patients undergoing induction therapy.[7]

We conclude that the incorporation of cladribine into the induction regimen containing daunorubicin and cytarabine does not provide additional risk for infections in newly diagnosed AML patients, including the incidence and spectrum of etiological factors as well as their severity and infection outcome, especially in the context of the duration of anti-infectious and supportive therapy. However, taking into account the retrospective nature of this study and relatively low numbers of cases analyzed, a prospective multicenter study is required to confirm our results. The precise analysis of the spectrum of infections as well as microorganisms responsible for febrile episodes may help in the prevention and therapeutic management of infectious complications in AML patients.

Conflict of interest: No conflict of interest to declare.

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